

RTR tetramer) serves as a control for the tetramer core and as a control for the RTR sequence. The absence of inhibitory activity from the ASA tetramer proves that the polylysine core and another hydrophobic tripeptide sequence is not active. These results show that the RTR peptide is specific for N-acetyl-PGP, given that the ASA sequence fails to block N-acetyl-PGP activation of polymorphonuclear leukocyte polarization.

The affinity of the RTR tetrameric peptide for N-acetyl-PGP was 10,000-fold greater than the RTR monomer. It is possible that the high charge distribution on the multimeric peptide, resulting from the arginine side chains, reduces the number of RTR conformations in the tetramer as compared to the RTR monomer.

The presence of favorable binding conformers in this limited number of conformations would increase the binding affinity with the chemoattractant. Alternately, or in addition, the close association of the RTR branches in the tetramer might limit the diffusion parameters of N-acetyl-PGP, keeping the chemoattractant in closer association with the tetramer and changing the kinetics of the interaction. These findings are consistent with an increasing effect as the multimeric structure increases the number of RTR sequences.

Peptides comprised of L-amino acids are rapidly degraded by enzymes in the body, especially in inflamed tissues. The D-form of complementary peptides has been shown to retain the biological activity of the L-form,<sup>18,19,29</sup> yet is more resistant to proteases and therefor more stable *in vivo*. For this reason the RTR tetramer, D-forms of all amino acids, may be an alternative therapeutic agent.

The implications of the methods of the present invention for the treatment of patients with alkali-injury of the eye might be substantial. If an inhibitor of polymorphonuclear leukocyte chemotaxis is administered immediately after an injury, the stimulus for polymorphonuclear leukocyte invasion into the cornea might be abrogated. Neutrophils constitute one of the most serious dangers to corneal integrity by initiating and perpetuating ulceration and causing perforation of the eye. If the initial recruitment of polymorphonuclear leukocytes by N-acetyl-PGP can be abolished or diminished, then other mediators, that are released from polymorphonuclear leukocytes, would not have an opportunity to magnify this neutrophilic response. The end result is preservation of

the corneal stroma, maintaining globe integrity and providing a suitable substratum upon which epithelial regrowth is encouraged.

Discovery of the alkali-generated chemoattractant has lead to a fuller understanding of chemotaxis in alkali-injuries and might also give rise to useful treatment in other eye diseases and in inflammation in other tissues of the body. For example, when soft tissue is injured by alkali anywhere in the body presumably the same chemoattractant is generated which would participate in triggering the inflammatory cycle initiated by alkali in these tissues. An example of this was observed with a variety of human blood components which elicited a polymorphonuclear leukocyte polarization response after exposure to alkali. It is conceivable that this genre of inhibitors might act to blunt the polymorphonuclear leukocyte inflammatory response in other unrelated, non-traumatic diseases where the chemoattractant is the same or sufficiently similar.

The use of this new methodology substantially shortens the time course for development of lead compounds; reducing the iterative approach in both traditional and computer molecular modeling techniques. This antisense technology might hold a key to